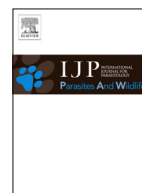


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Brief Report

Trichinella species circulating in wild boar (*Sus scrofa*) populations in PolandEwa Bilska-Zajac^a, Mirosław Różycki^{a,*}, Ewa Chmurzyńska^a, Gianluca Marucci^b, Tomasz Cencek^a, Jacek Karamon^a, Łukasz Bocian^c^a Department of Parasitology and Invasive Diseases, National Veterinary Research Institute in Pulawy, Al. Partyzantów 57, 24-100 Pulawy, Poland^b Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy^c Department of Epidemiology and Risk Assessment, National Veterinary Research Institute Pulawy, Al. Partyzantów 57, 24-100 Pulawy, Poland

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ABSTRACT

Hunting in Poland has a long tradition and became more popular after 1990. Each year over 60,000 wild boar are hunted. Some of them may act as *Trichinella* carriers thus all carcasses of wild boar are systematically sampled in game-handling establishments as part of the post-mortem examination. The aim of the study was to determine the species of *Trichinella* and to evaluate the year to year differences in the occurrence of those species in the populations of wild boar in Poland. Samples for the study were provided by the Veterinary Inspection Service. Wild boar carcasses were examined using a digestion method. Only samples recognized as positive for *Trichinella* in these examinations were sent to the National Reference Laboratory (NRL) for confirmation of genus identity. Samples from 450 animals were obtained for the study (380 muscle samples and 70 larval isolates preserved in 90% ethyl alcohol). Tissue samples were digested to isolate larvae. Extracted larval DNA was amplified using a modified multiplex PCR protocol to identify the species of *Trichinella*. Five larvae from each sample were examined by PCR. The study revealed that *Trichinella spiralis* and *Trichinella britovi* are present in wild boar in Poland in a ratio of 3:1. Mixed infections with *T. spiralis* and *T. britovi* were found in 1% of the animals.

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1. Introduction

The *Trichinella* genus (class Adenophorea, order Trichocephalida, family Trichinellidae) includes nine species so far: *Trichinella spiralis*, *Trichinella britovi*, *Trichinella nativa*, *Trichinella nelsoni*, *Trichinella murrelli*, *Trichinella zimbabwensis*, *Trichinella papuae*, *Trichinella pseudospiralis*, *Trichinella patagoniensis*, and three genotypes: T6, T8, T9. Four of the species, *T. spiralis*, *T. britovi*, *T. nativa* and *T. pseudospiralis*, are known to be circulating in Europe. Transmission of the parasite occurs when a susceptible host ingests muscle tissue containing live *Trichinella* larvae.

Humans become infected with *Trichinella* by eating raw or undercooked pork, horse or game meat. The disease usually manifests itself with non-specific symptoms which often resemble those of a cold or food poisoning, and diagnosis is sometimes difficult. In severe cases of trichinellosis, death may also occur. Previous research shows that wild boar and pig meat are the most

common sources of human infection in Poland. Based on data obtained from the National Institute of Public Health – National Institute of Hygiene (NIPH – NIH) in the years 2000–2010, 932 cases of human trichinellosis were reported in Poland (Cabaj, 2006; Sadkowska-Todys and Gołab, 2007; Bilska-Zajac et al., 2011). For most of these, the source of infection was wild boar meat (http://www.pzh.gov.pl/oldpage/epimeld/index_p.html, 02/27/2013). Previous data obtained in a very limited study (69 samples) indicated the circulation of *T. spiralis* and *T. britovi* in wild boar in Poland (Cabaj, 2006). In samples examined in 2008–2009 the ratio of *T. spiralis* to *T. britovi* in wild boar populations was assessed as 6 to 1 (Różycki et al., 2010). The aim of the current study was to determine the species of *Trichinella* present in the populations of wild boar in Poland and to assess the year to year differences in the *Trichinella* species occurring.

2. Materials and methods

Samples for the study were provided by the Veterinary Inspection Service (VIS). All wild boar carcasses (over 60,000 yearly) were examined according to the European Commission Regulation 2075/2005, Annex I, Chapter III. Samples recognized as positive for *Trichinella* in VIS laboratories were sent to the National

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Reference Laboratory (NRL) for genus identification. Samples were obtained as frozen muscles or as larvae preserved in alcohol. *Trichinella*-positive samples were collected from all regions of Poland from 2009 to 2012. Overall, 450 samples from *Trichinella*-positive wild boar were examined: 380 frozen muscle samples and 70 larval isolates (in 90% ethyl alcohol). Muscle samples weighing 50 g were digested to isolate *Trichinella* larvae. Larvae obtained from these digests were preserved in 90% ethyl alcohol for further examination by PCR.

The second type of samples, larval isolates, were examined under a stereomicroscope by skilled personnel to confirm their identity as *Trichinella* larvae (on the basis of morphological characteristics). *Trichinella* larvae were retrieved from the sample for further DNA extraction. Larval DNA was extracted and purified by DNA IQ™ System kit (Promega, USA) according to the protocol of the European Reference Laboratory for Parasites (EURLP) for identification of *Trichinella* muscle stage larvae at the species level (<http://www.iss.it/binary/crlp/cont/PCR%20method%20WEB%20SITE.1177083731.pdf>, 02/13/2012). A pool of five larvae from each sample was analyzed. For PCR reactions, a modified version of the EURLP Multiplex PCR protocol was used. The protocol was modified by using only two pairs of primers and a lower annealing temperature. The following primers were used: CPIF (5'GT TCCATGTGAACAGCAG3') and CPIR (5'CGAAAACATACGACAAC TGC3'), which amplified the expansion of segment five (ESV) region in all known *Trichinella* species and genotypes, and CPIIF (5'GCTACATCCTGATCTGTT3') and CPIIR (5'AGACACAATATCAACCA-CAGTACA3'), which amplify a partial region of ITS1 and that are specific for *T. britovi*. PCR reactions were performed in a 50 µl volume containing 3 mM MgCl₂, 200 µM each dNTP, 1 U Taq Polymerase (Fermentas) and 0.1 µM of each primer. Amplification conditions consisted of 36 cycles as follows: denaturation at 95 °C for 40 s, annealing at 50 °C for 30 s and extension at 72 °C for 30 s. An initial denaturation was performed at 95 °C for 4 min and a final extension at 72 °C for 3 min.

Products of all PCRs were separated with gel electrophoresis using a 1.5% agarose gel and stained with ethidium bromide. The bands in the gel were visualized and photographed under UV light.

Trichinella isolates collected in 2009–2011 were sent for confirmation to EURLP, where five single larvae from each animal were examined separately according to the EURLP protocol. The significance of year to year changes in prevalence of the species detected were evaluated by a trend analysis (Statistica 10; StatSoft, Poland). Data obtained were incorporated in a map of *Trichinella* species location by province (QGIS ver. 1.7.4).

3. Results and discussion

The result of this study confirm the presence of *T. spiralis* and *T. britovi* in wild boar populations in Poland. PCR products specific for *T. spiralis* (173 bp) and *T. britovi* (127 and 253 bp) were obtained for 424 samples (94.2%) of 450, for 26 insufficient DNA was recovered, or the samples had degraded during storage (Fig. 1). The PCR-positive isolates included: 55 samples collected in 2009, 214 samples collected in 2010, 112 samples collected in 2011, and 43 samples collected in 2012. Of 424 *Trichinella* isolates tested positive by multiplex PCR, 319 (75.2%) were identified as *T. spiralis*, 101 (23.8%) as *T. britovi*, and 4 isolates (1%) as mixed infections of *T. spiralis* and *T. britovi* (Table 1).

The data obtained showed that *T. spiralis* and *T. britovi* are present in wild boar in Poland in a ratio of 3:1. The species of the larvae collected from 2009 to 2011 were confirmed by EURLP. Samples from 2012 were examined in-house but not yet confirmed by EURLP. This surveillance study on *Trichinella* species prevalence in wild boar indicates that the ratio of samples containing *T. spiralis* and *T.*

britovi species is slightly changing year by year. However, these differences were not statistically significant (*T. spiralis*: $F = 0.64$; $p = 0.42$; *T. britovi*: $F = 0.71$; $p = 0.40$). The geographic distribution of the *Trichinella* species is shown in Fig. 2.

The purpose of the present study was to identify the species of *Trichinella* infecting wild boar in Poland. In order to assess the epidemiological situation for *Trichinella* in Poland two pairs of primers were used that allow the identification of the *Trichinella* species known to be circulating in Europe (i.e., *T. spiralis*, *T. britovi*, *T. pseudospiralis* and *T. nativa*).

Two *Trichinella* species (*T. spiralis* and *T. britovi*) were found to be present in wild boar populations in Poland. These species are also found in bordering countries, but their ratio differs from country to country. For example, species identification of larvae from wild boar in the Czech Republic and Slovakia showed only *T. britovi* (Pozio, 2007; Hurnikova and Dubinsky, 2009). In wild boars from Poland and Germany the ratios of *T. spiralis* to *T. britovi* were similar (Pannwitz et al., 2010). *T. pseudospiralis* infections have been reported in wild boar in Germany (Nockler et al., 2006), Italy, France, Sweden, Finland and the Netherlands (Merialdi et al., 2011), but not in Poland. *T. nativa* is an arctic species known to infect carnivores, including wolves, foxes, lynx and raccoon dogs at high latitudes. This species has also been reported in wild boar: three times in Latvia and Estonia, and once in Lithuania and Finland (<http://www.iss.it/site/Trichinella/scripts/reso.asp>, 02/27/2013). All those cases were in Baltic countries. In Poland, until now, there has been no report of *T. nativa* or *T. pseudospiralis* in wild boar, but *T. nativa* has been detected in red fox (Chmurzyńska et al., in press).

The present study has shown that the prevalence of *T. spiralis* (75.2%) in infected wild boar in Poland is higher than *T. britovi* (23.8%). The 3:1 ratio of these species was similar to that observed in wild boar in Germany, according to data from the International Trichinella Reference Center (ITRC). A similar ratio (3.5:1) was observed in Spain (Serrano et al., 1998). In contrast, the reported ratios in France and Lithuania are 1:13 and 1:3, respectively (<http://www.iss.it/site/Trichinella/scripts/reso.asp>, 02/27/2013).

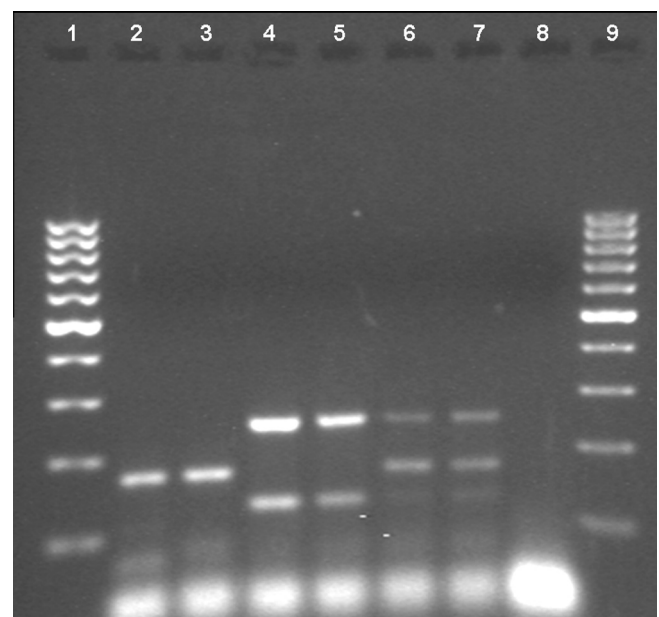


Fig. 1. Example of electrophoretic patterns obtained from multiplex PCR on *Trichinella* larvae collected from wild boar. Lane 1 and 8 molecular weight marker (Fermentas 100 bp DNA Ladder); lanes 2 and 4, *T. spiralis*; lanes 3 and 5, *T. britovi*; lane 6, *T. spiralis* and *T. britovi* mixed infection; lane 7, negative control.

Table 1
Number and prevalence (%) of *Trichinella*-positive samples detected, 2009 – 2012.

Species	2009		2010		2011		2012		Overall	
	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%	No. of positive samples	%
<i>T. spiralis</i>	40	72.7	159	74.3	86	76.8	34	79	319	75.2
<i>T. britovi</i>	15	27.3	53	24.8	24	21.4	9	21	101	23.8
Coinfection <i>T. spiralis</i> and <i>T. britovi</i>	0	0	2	0.9	2	1.8	0	0	4	1

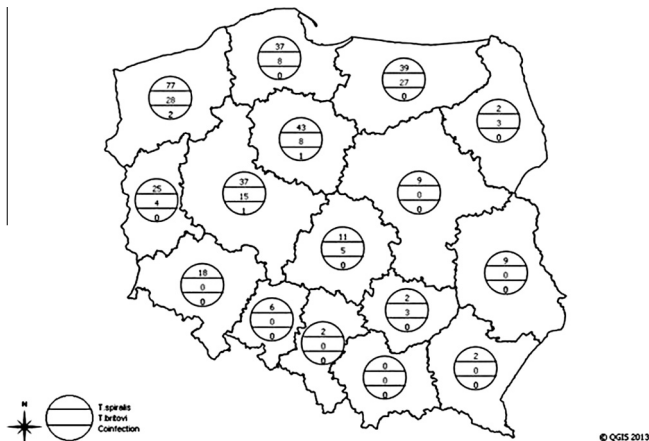


Fig. 2. Geographical distribution of *Trichinella* species occurs in wild boar populations in Poland.

The current study also confirmed that coinfections with *T. spiralis* and *T. britovi* are possible in wild boar in Poland, in this case in 1% of the samples examined. These coinfections have also been reported in 3.2% and 5.0% of wild boar in Spain (Serrano et al., 1998; Perez-Martin et al., 2000).

Coinfections may also involve other species of *Trichinella*, for example *T. spiralis* and *T. pseudospiralis* in wild boar in Germany (Nockler et al., 2006).

The results of our study showed a moderate increase in prevalence of *T. spiralis* in wild boar (from 72.7% in 2009 to 79% in 2012), while there was a slight decrease for *T. britovi* (from 27.3% to 21%). These trends are not, however statistically significant. This might be a consequence of low number of samples tested or to differences in the number of samples tested each year. Therefore, these findings require further investigation.

In conclusion, the presence of *Trichinella* spp. in wild boar in Poland remains a great public health problem. The fact that most human trichinellosis cases in Poland were attributed to the consumption of wild boar meat in the past especially emphasizes the importance of preventive measures, most importantly the systematic inspection of wild boar for *Trichinella*. This and other preventive measures are of great importance, particularly in regions of the country where raw meat products are consumed.

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